Turning cold tumors into hot tumors: harnessing the potential of tumor immunity using nanoparticles

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TURNING COLD TUMORS INTO HOT TUMORS: HARNESSING THE POTENTIAL OF TUMOR IMMUNITY USING NANOPARTICLES

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Abstract

Introduction: Immune checkpoint inhibitors have considerably changed the landscape in oncology. However apart world-acclaimed success stories limited to melanoma and lung cancer, many solid tumors failed to respond to immune checkpoint inhibitors, due to limited immunogenicity, unfavorable tumor micro-environment (TME), lack of infiltrating T lymphocytes or increase in Tregs.

Areas Covered: Combinatorial strategies is foreseen as the future of immunotherapy, and using cytotoxics or modulating agents is expected to boost the efficacy of immune checkpoint inhibitors. In this respect, nanoparticles displaying unique pharmacokinetic features such as tumor targeting properties, optimal payload delivery and long-lasting interferences with TME, are promising candidates for such combinations. This review covers the basis, expectancies, limits and pitfalls of future combination between nanoparticles and immune check point inhibitors.

Expert Opinion: Nanoparticles allow optimal delivery of variety of payloads in tumors while sparing healthy tissue, thus triggering immunogenic cell death. Depleting tumor stroma could further help immune cells and monoclonal antibodies to better circulate in the TME, plus immune-modulating properties of the charged cytotoxics. Finally, nanoparticles themselves present immunogenicity and antigenicity likely to boost immune response at the tumor level.

Keywords: Immunotherapy; nanoparticles; drug delivery; immunogenicity; pharmacokinetics; cancer

1. Current achievements and Limits of immunotherapies

The use of immune checkpoint inhibitors has profoundly changed the evolution profile of cancer patients with advanced disease [1]. Antibodies targeting the T cell inhibitory receptors cytotoxic T-lymphocyte antigen 4 (CTL A 4) and the programmed death receptor 1 (PD 1) (or its ligand PDL 1) are now clinically recognized with FDA approvals in melanoma, non-small-cell lung cancer (NSCLC), renal cell carcinoma and head and neck cancers to name but a few [2]. Checkpoint inhibitors have a different toxicity profile than chemotherapy with a lower incidence of pancytopenia or digestive toxicities which are the most frequent adverse events with chemotherapeutic agents. However initial over-optimism about checkpoint inhibitors has been tempered by the limited number of patients exhibiting spectacular response rates, or the issue of emerging specific toxicities [1]. Another limitation is the fact that baseline biomarker studies have shown that checkpoint blockade therapy is mainly active through reactivation of antigen specific T cells present within tumor micro-environment (TME) [2]. It is a matter of fact that the majority of solid tumors do not exhibit a productive T cell infiltrate and can thus be considered as non-T cell-inflamed [2]. Thus turning cold tumors into hot tumors is currently one of the major goals to improve treatment of solid tumors with checkpoint inhibitors.

2. Turning cold tumors into hot tumors: an ongoing challenge

To achieve transforming cold tumors into hot ones, the wide range of possible combinations between immunotherapeutic agents and other drugs has to be appraised. A recent review by Lafolla and coworkers [3] covered a total of 410 combination trials involving two or more than two immune-oncology drugs, mostly in skin and genitourinary malignancies. Gene expression data from TCGA were investigated and put into evidence 178 targets in 9089 tumors from 19 cancer-types. This allowed several promising new drug combinations to be identified. Above all, the impact of a combination between checkpoint inhibitors with cytotoxics or targeted agents to reshape tumor milieu in order to get immune infiltrate compatible with an expected activity of the checkpoint

inhibitors is getting more and more attention [4]. The main contributor for a replenishment of cytotoxic T cells is the tumoral neo-epitope production [5]. This neo-epitope production can be stimulated in different way, such as co-administration of agents, the most efficient ones being standard cytotoxic drugs (5). Modulation of the immune response through checkpoint inhibition may be increased by cytotoxic chemotherapy not only through the potential for neo-antigen cross-presentation but also by inhibiting the ratio of cytotoxic lymphocytes to T regulatory (Tregs) lymphocytes [6] or blocking the STAT6 pathway to upregulate dendritic-cell activity [7].

3. Nanoparticles at a glance

Nanoparticles are small (i.e., <200 nm) entities designed to cargo anticancer agents throughout the body to the tumor site. Beyond canonical liposomes, a wide range of supports and scaffolds have been made available now, ranging from dendrimers, squalene derivatives, nanospheres, to inorganic carriers such as silica beads or gold nanoparticles [8]. Despite their heterogeneity (Figure 1), all these nanoparticles share common features, such as ability to passively target tumor tissue and to accumulate preferentially in the tumor micro-environment, through the Enhanced Permeation and Retention effect (EPR effect). Indeed, provided that their size is below 200 nm, nanoparticles will pass through gaps and loose tight junctions of the tumor neovessels, thus enabling a preferential extravasation in tumor neighborhood. In addition, the latest generation of nanoparticles such as immunoliposomes or conjugated nanoparticles can further target actively tumor antigens or tumor micro-environment [9]. Overall, nanomedicines in oncology all aim at improving the efficacy/toxicity balance of cytotoxics through an optimized biodistribution profile sparing healthy tissues and targeting tumor cells (Figure 2) [10]. In addition to carrying drugs, nanoparticles can be further used in theranostic applications such as iron-oxyde constructs [11], or radiosensitizing agents [12], highlighting how the carrier itself could have therapeutic applications. This *per se* efficacy could come from intrinsic properties of inorganic particles, such as antibody-dependent cell cytotoxicity

(ADCC) – even when < 200 nm, nanoparticles are large enough to trigger immunogenic response, especially when they are composed of inorganic materials [13].

4. How nanoparticles could help to preserve patient's innate immunity while delivering cytotoxics.

Cancer patients are generally heavily treated by a wide variety of drugs but standard cytotoxics remain the backbone of most regimens in solid tumors. The canonical and common drug-related toxicity of most cytotoxics is pancytopenia, leading to sepsis and sometimes lethal outcome in the most dramatic cases [14]. This side-effect is the direct consequence of the non-specific mechanism of action of the vast majority of cytotoxics, which have all been selected to interfere with rapidly-dividing cells such as cancer cells. Because hematopoietic progenitors are also rapidly-dividing cells, they are doomed to be affected by most chemotherapy regimen, regardless of the pharmacological class (e.g., antimicrotubules, antimetabolites, or alkylating agents to name but a few). In the light of the critical importance of innate immunity against cancer, one can assume that affecting lymphocytes count or other cells implicated in the immune response will have deleterious effects on the clinical outcome first, and will impair the efficacy of any immunotherapy-based strategy next, thus triggering innate resistance. For instance, several studies have suggested that elevated neutrophil-to-lymphocyte ratios (i.e., NLR >5) or diminished absolute lymphocyte count are associated with poor clinical outcome in patients treated with immune check point inhibitors [15,16]. It has already been shown that prior treatment with cytotoxics and history of drug-related lymphopenia are associated with poor prognosis of various cancer types [17,18] and considered now as a possible unfavorable factor in patients treated with immune check point inhibitors [19]. This hypothesis is however controversial and other studies have failed to confirm this relationship, at least during phase-I studies [20]. Indeed, transient lymphopenia can be an opportunity for reshaping the repertoire of immune cells [21], thus explaining why an in-depth understanding of the duration

and level of chemo-induced lymphopenia plus full characterization of the kinetics of T-cells recovery is critical to better picture the impact of lymphopenia on homeostatic proliferation and efficacy of immune check-point inhibitors. As seen before, one of the most remarkable achievement of nanoparticles is their ability to target tumor tissue, thus sparing, at least partly, healthy cells and reducing thereby canonical cytotoxics-related side effects such as hematological toxicities. Several experimental data showed how the biodistribution of anticancer agents shifts from totally non-specific when used as free drugs to a more tumor-directed distribution when used as nanoparticles. For instance, tumor *VS.* healthy tissue comparative biodistribution studies showed that paclitaxel is more concentrated in tumors and less in liver, spleen or kidneys when administered as nab-paclitaxel, a 120 nm conjugated form of paclitaxel linked to a bioengineered albumin that targets SPARC and other glycoproteins of the TME [22]. As a direct consequence of this increase in tumor-targeting properties, several studies have shown how nanoparticles led to reducing side effects in patients scheduled for chemotherapy. Because one of the canonical treatment-related severe toxicity is pancytopenia, sparing hematopoietic progenitors could help to maintain patient's innate immune system functional. For instance, liposomal doxorubicine or liposomal vincristine show both reduced toxicities as compared with free doxorubicine or free vincristine [23,24]. In a non-clinical study, it has been demonstrated that stealth liposomal 5-FU was less likely to trigger severe leucopenia than standard 5-FU [25]. Similarly, severe neutropenia appeared less in patients treated with nab-paclitaxel drug conjugate, as compared with standard paclitaxel [26], and model-informed studies have further confirmed how paclitaxel nanoparticles were less toxic against blood cells [27]. More recently, in the Napoli-1 trial, liposomal irinotecan associated with LV-5-FU led to 18% of severe hematological toxicities, a figure markedly lower than the incidence of this toxicity when standard irinotecan is associated with 5-FU, such as the FolFiri regimen [28]. Finally, by sparing hematologic progenitors, nanoparticles can be indirectly further helpful in patients scheduled for immunotherapy, by reducing the need to use antibiotics in patients. Indeed reducing toxicities, especially the neutropenia-related infections, will lead to cutting broad-spectrum antibiotherapy,

whom administration has been repeatedly associated with reduced survival in immunotherapy-treated patients [29], much probably because of the disruption of gut microbiome necessary to the immune surveillance, e.g. by enhancing the function of dendritic cells and elevating recruitment and function of T cells interacting with these later [30]. Overall, because they are better tolerated, using nanoparticles to deliver anticancer agents could be a promising strategy when a patient is scheduled next for immunotherapy because it is more protective towards patient's immunity, both by reducing the incidence of hematological toxicities and by limiting antibiotics whom use has been proven to be deleterious on survival.

5. How Nanoparticles could increase neo-antigens burden and modulate actors of immune response.

Standard anticancer agents are expected to boost immunotherapy at least in two distinct ways: by triggering immunogenic cell death and by adding effects on cancer and normal host cells in the TME.

5.1. Delivering immunogenic agents.

Lack of suitable neo-antigens and disruption in antigen processing or presentation is usually associated with impaired immune response to cancer [31]. Consequently, promoting immunogenic cell death prior introducing immune check-point inhibitors should help improving efficacy, supporting the hypothesis that cytotoxics should be associated indeed with immunotherapy [32,33]. Immunogenic response induced by cytotoxics probably involves the purinergic receptor P2RX7 or the pattern recognition receptor toll-like receptor-4 (TLR-4) [34]. In addition, intrinsic tumor immunogenicity can be enhanced with cytotoxics by upregulating tumor antigens such as CEA or by increasing tumor antigen presentation and recruitment of antigen presenting cells (APCs), i.e. through the overexpression of MHC class-I molecules. Using nanoparticles should further trigger immunogenic response, because they frequently exhibit higher cytotoxicity as compared with standard drugs. For instance, liposomal 5-FU shows greater antitumor properties, both in vitro and in vivo, as compared with free 5-FU. In particular, stealth liposomal 5-FU induces deeper TS inhibition in

colorectal cell lines, thus triggering Fas-mediated apoptosis because of the thymineless stress in cancer cells [35]. In a quite similar way, liposomal gemcitabine proved to perform better than standard gemcitabine in pancreatic cancer models [36], and nab-paclitaxel exhibited higher antiproliferative efficacy than free paclitaxel in a variety of solid tumors [22]. The superior antiproliferative efficacy of nanoparticles over standard cytotoxics has been confirmed at bedside as well, since liposomal vincristine was found to be more effective than standard vincristine [24] whereas the combo daunorubicine + cytarabine given as a liposomal formulation improved response rates as compared with standard combination in patients with myelodysplastic syndromes [37]. In addition to the selective delivery of cytotoxics likely to trigger immunogenic cell death, nanoparticles can be further used to deliver antigens or adjuvants to specifically dendritic cells (DCs). For instance, lymph node-targeting nanoparticle-conjugate vaccines (i.e., TAA-NP and CpG-NP) proved to induce stronger cytotoxic CD8+ T-cell responses, higher antiproliferative efficacy and extended survival in mice bearing melanoma [38].

5.2. Modulating TME Immune cells.

In addition to promoting cancer immunogenicity by increasing neo-antigens burden, nanoparticles carrying cytotoxics or cytokines could modulate as well immune response through a variety of mechanisms, ranging from upregulating the expression of MHC Class I molecules to which the antigens bind, upregulating of co-stimulatory molecules and PD1/PDL1 expression, to downregulating co-inhibitory molecules such as PD-L1/B7-H1 or B7-H4, thus enhancing the strength of effector T cell activity. For instance, canonical 5-FU can abrogate Myeloid Derived Suppressive Cells (MDSCs) and Treg activity, while additionally it makes T cell-mediated lysis more effective through Fas-dependent mechanisms [39–41]. Cisplatin and cyclophosphamide both proved to decrease Tregs expression and to increase CD8+ T cell activity [42]. In addition to modulating T lymphocytes, chemotherapy, especially when administered with low dose such as following

metronomic regimen, can modulate as well DCs phenotype and function. For instance vinca-alkaloids, taxanes and cyclophosphamide proved to enhance the function of DCs, much probably through an IL-12 dependent mechanism [43,44]. Much interestingly, the very distribution profile of nanoparticles, which are expected to nest in the TME and start releasing their payload regularly and over a long period of time, could meet this metronomic-like exposure required to enhance DC. One of the common features of all drug-carriers is indeed to stay longer in the tumor site, whereas free drugs enter massively before being rapidly cleared out of the tumor tissue. Consequently pharmacodynamic properties of standard cytotoxics are best described by the Hill equation, i.e. the higher the dose, the higher the effect on tumor cells or TME, with no lag compartment when modeling the effects. In contrast, the complex interplay between carrier's PK, payload release, carrier's interactions with TME, makes the PK/PD profiles of nanoparticles more likely to ensure a sustained and constant release of cytotoxics at the tumor site [10]. In addition and because of the complete lack of specificity of anticancer agents, the high dose regimen required to trigger immunogenic cell death will have deleterious impact on bone marrow cells (see previous paragraph), with subsequent lack of clonal expansion of T cells. This calls for using nanoparticles to redefine optimal dosing and scheduling of cytotoxics, so as to limit their hematological toxicities while preserving immunogenic cell death at the tumor level. In addition, drastic tumor debulking with cytotoxics leading to minimal residual disease may mitigate the negative impact of tumor burden on the efficacy of immune check point inhibitors. In this context, using nanoparticles once again could help to solve this once contradictory issue, because PK/PD relationships of nanoparticles allow sparing healthy cells while exerting maximal antiproliferative efficacy at the tumor level [10]. Although published data remain sparse, it has been recently confirmed that nanoparticles could achieve higher antiproliferative efficacy when combined to immune check point inhibitors because of their immunomodulating properties. For instance after having demonstrated how topoisomerase-I inhibitors such as irinotecan could enhance T-cell-mediated cytotoxicity of melanoma tumors in vitro, liposomal irinotecan was associated with anti-PDL1 in tumor-brearing-mice. Results confirmed

greater efficacy of this association, much probably because of the higher cytotoxic functionality observed with CD8+ T cells in the combination group [45].

5.3. Triggering immunogenic response.

Nanocarriers themselves can exert some kind of immunomodulation on their own, due to their physico-chemical composition. Despite being < 200 nm, nanoparticles remain foreign objects likely to be recognized by the MPS. When circulating in the body, this could be an issue associated with lack of stability and different strategies can be undertaken to increase nanoparticle stealthness, such as surface pegylation [46]. However once the TME is reached, immunogenicity and antigenicity of nanoparticles could help to boost immune response against cancer [47]. Generating antibodies against nanoparticles can be obtained through a thymus-dependent pattern. Bioengineered nanoparticles will activate DC's producing cytokines that activate T-helper cells. These T-helpers will recognize next antigens from APCs and finally prompt B cells to proliferate and to differentiate against the antigenic nanoparticles. In addition, B-cell activation can be achieved by repetitive elements in the antigen without T-cell involvement [48]. Of note, beyond inorganic scaffolds such as metal-based nanoparticles [49], it has been shown that antibodies can be raised even against 100% biocompatible lipid carriers such as liposomes [50,51]. Of note, even surface pegylation, a common strategy in nanomedicine to increase stealthness and mask nanoparticles from scavengers and phagocytic cells, can show some immunogenicity as anti-PEG IgM have been already described in non-clinical models [52]. Nanoparticles immunogenicity could be therefore mostly based upon antibody-dependent cell cytotoxicity (ADCC)-like mechanism, the antibodies being capable to recognize either surface groups or core components. Because in oncology nanoparticles are expected to preferentially accumulate and concentrate in tumors, this immunogenicity could contribute to attract in TME new actors of the immune response. However, to what ADCC could contribute to a better efficacy when using next immune check point inhibitors must be clearly investigated, as some nanoparticles showed inconsistent results. For instance, silicon nanoparticles encapsulated with

sorafenib and coated with anti-CD326 antibodies showed increased efficacy in various breast cancer models. However, concentration-dependent ADCC was evidenced in MCF7 cells, but not in the MDA-MB231 model [53], whereas elsewhere a complete lack of ADCC mechanism was reported with gold nanoparticles [54]. Despite this discrepancy in ADCC properties, several strategies have been successfully tested to boost patient's immunity. For instance, inorganic nanoparticles (i.e., polymeric or gold) with surface modifications were used to target Tumor Associated Macrophages (TAM) thanks to PEG-sheddable, mannose-modified constructs [55]. Elsewhere, artificial APCs for direct CTL activation were achieved using iron-oxide nanoparticles coated with dextran and quantum dots coated with avidin led to an increase in T-cells and successful tumor rejection in melanoma-bearing mice [56].

6. Helping immune cell and therapeutic monoclonal antibodies to better trafficking into tumor micro-environment

Tumor-associated immune factors and chemokines in TME are heterogeneous and play a critical role in determining clinical outcome in cancer patients treated with immune checkpoint inhibitors. As early of the early 2000's it was found that TME was associated with higher turnover of T-lymphocytes, as compared with non-cancerous tissue, suggesting that targeting TME could be of importance in the era of immunotherapy because of its immunosuppressive properties [57]. By affecting TME cytotoxics may enhance the efficacy of tumor-educated lymphocytes. The higher the amount of cytotoxics in tumor surrounding, the more effective will be the action on tumor stroma, desmoplastic cells, fibroblasts and eventually tumor cells. However, it seems that the balance between direct cytotoxicity (and subsequent immunogenic cell death), depleting effect on TME, and immunomodulating properties of cytotoxics all depends on the dosing and the scheduling of the regimen, and should be finely tuned as it is probably drug-dependent. It has been demonstrated, long before immunotherapy was on the rise, that neo-adjuvant paclitaxel could ease new

intratumoral immune cell infiltrates with subsequent higher apoptotic response and clinical efficacy in breast cancer [58]. Much interestingly, paclitaxel can be delivered either as a cremophor formulated drug, or as a conjugated nanoparticle, nab-paclitaxel. Nab-paclitaxel proved to deplete tumor stroma, reducing density and disrupting tumor micro-environment, in addition to direct effect on tumor cells microtubules. Because one of the main causes for immune escape is unfavorable TME [59], such additional mechanism of action is likely to make hot an initially cold tumor, thus further easing the infiltration of dendritic cells and antigen-experienced T cells as compared with free paclitaxel. Indeed, tumor-specific CD8 T-cells subsequently differentiated into effector T-cells require trafficking to the TME prior to kill cancer cells expressing neoantigens. By depleting tumor stroma, nanoparticles could actually both enhance the infiltration in TME of T cells, but as well that of therapeutic monoclonal antibodies such as immune checkpoint inhibitors which are usually molecules too large to properly reach solid tumors because of high stroma density and binding-site barrier issues [60]. Although to date no experimental data are available to support this hypothesis because little is known on the tumor distribution of immune check-point inhibitors *in vivo*, one can speculate that depleting effect on TME by nanoparticles should additionally increase drug delivery of these large therapeutic antibodies next.

7. Conclusion: How nanoparticles could be best combined with immunotherapy.

Overall, nanoparticles can be used to boost immunotherapy in several ways. Optimizing delivery at the tumor site of a variety of payloads ranging from cytotoxics triggering immunogenic cell death, cancer vaccine or therapeutic RNAs is an appealing strategy. Interestingly, nanotechnologies offer today such a wide sort of scaffolds that there is no limit but the imagination of the researchers to achieve this goal (Figure 2). For instance gold nanoparticles have been designed to deliver anti-VEGF siRNA both to TAMs and to lung cancer cells, achieving a synergistic efficacy in tumor-bearing mice [61]. Elsewhere, dendrimers loaded with paclitaxel conjugated with mAbK1 antibodies proved to be

effective in ovarian cancer mice models [62]. Nontoxic core-shell nanoparticles encapsulating photosensitizer pyrolipid proved to increase the efficacy of anti-PDL1 in metastatic breast cancer models, partly by increasing tumor mutational burden after photodynamic therapy [63]. In breast cancer model again, liposomal nanoparticle coated with tumor-targeting peptide and co-encapsulating anti-PI3K drug plus a specific agonist of therapeutic T cells, proved to act synergistically when CAR-T cells were administered next. Increase of immune effector cells such as CD8+ lymphocytes and invariant natural killer cells were evidenced, and increase in efficacy was further confirmed in a mouse model of human glioblastoma [64]. Elsewhere nanogels carrying interleukin-15 were successfully developed to boost mouse T cell and CAR-T cell therapy in a melanoma model [65]. In addition to drug delivery properties or tumor micro-environment reshaping, nanoparticles can also exhibit directly some immunogenic properties. Size, composition, surface properties, electric charge, all probably matter and call for a comprehensive understanding of the immunogenicity and antigenicity of nanoparticles. For instance, cobalt oxide nanoparticles coated with PMIDA and conjugated to a lysate antigen triggered increase in IgG and CD4+ response in mice [66]. In breast cancer models, manganese dioxide liposomal nanoparticles combined with doxorubicin increased the infiltration of CD8+ T cells in TME, thus boosting antitumor efficacy as compared with free doxorubicine alone. Much interestingly, naïve tumor-bearing mice transplanted with splenocytes from mice previously treated by this combination were able to control tumor growth, whereas splenocytes from control mice or from doxorubicine-treated mice had no antiproliferative effect, thus highlighting how immunomodulating properties of nanoparticles were at the origin of the observed efficacy [67]. Although only experimental, all these studies pave the way for future combinations with immune check point inhibitors. However, there are several pitfalls in developing combinatorial strategies between nanoparticles and immunotherapy. First, the most immunogenic nanoparticles such as the ones based upon metal, silica nanosphere of other inorganic or polymeric components, have all unaddressed issues in terms of nanosafety, because little is known about their possible long-term effects once injected in the body [68]. In addition, being too immunogenic may

expose nanoparticles to early recognition by macrophages and scavenging cells before the nanoparticles even reach the tumor site. Second, apart long-studied liposomes, little is known about the exact pharmacokinetic profile of the most recent nanoparticles, because their unique features differ to that of standard drugs [10,46]. Extensive PK/PD modeling is therefore much awaited to better understand how and when nanoparticles should be best combined with immune check-point inhibitors. For instance, increasing mutational burden and achieving drastic tumor debulking is more likely to be observed when high concentrations of cytotoxics reach tumor cells, whereas immumodulating properties on DCs, Tregs or effector T-cells have been conversely reported with metronomic regimen leading to sustained and continuous exposure to low levels of drugs [69]. The era of trial-and-error strategies, inherited from the 20th century medicine, is gone and with respect to the possible number of combinations made available now, strong pharmacometric support is mandatory to ensure successful development of immuno-nano-therapeutics.

Expert Opinion:

Beyond the initial frenzy sparked by the successful stories in melanoma and lung cancer, immunotherapy seems to have reached a glass-ceiling in oncology, at least as single-agents. Poor trafficking of activated T lymphocytes to the tumors, massive recruitment of Tregs or MDSCs in the tumor micro-environment, low mutational burden, are the most frequent reasons explaining the innate resistance of most solid tumors towards immune checkpoint inhibitors. Pre-treating patients with agents likely to boost immune response is therefore an appealing strategy to optimize the efficacy of immunotherapy. Nanoparticles present a wide range of characteristics such as optimized drug delivery in the tumor surroundings and immunogenic properties that make them suitable candidates for combinatorial strategies with immune checkpoint inhibitors. In addition, nanoparticles can cargo a limitless number of payloads such as cytotoxics, cytokines, vaccines or nucleotides, all likely to modulate tumor immunity or to trigger immunogenic cell death. However, a current

bottleneck in the clinical development of nanoparticles remains scale-up issues and pitfalls when shifting from bench-size batches to clinical-size batches. Apart from liposomes and immunoliposomes for which proof-of-concepts of successful industrial production has already been made, the complexity to produce large and reproducible batches of sophisticated scaffolds has to be taken into account and could be a limitation to future bedside applications. Nanosafety issues and emerging tight regulations regarding manufacturing and handling of nanoparticles also has to be taken into account as a possible limitation, especially with inorganic materials [70]. In addition, there is another possible safety risk when administrating highly immunogenic nanoparticles in combination with immune check point inhibitors, because of a possible potentiation of immune-related adverse effects (IRAEs) reported with immunotherapy. In addition, immunogenic nanoparticles can also be recognized by scavenging cells or macrophages when trafficking in the blood, spleen or liver, thus having little chance to reach the tumor. Stealthness is a common strategy to limit spleen or liver uptake – however designing a nanoparticle smart enough to be stealth in healthy tissue to limit IRAEs while triggering immunogenicity only once malignant tissue are reached is particularly challenging. Finally, lessons have to be learned from previous recent failures when trying to combine empirically immune check-point inhibitors with other treatments such as metronomic chemotherapy, radiotherapy or anti-angiogenics. In-depth knowledge of nanoparticles PK/PD profiles, ideally using model-informed approaches, is urgently needed to better picture the optimal modality to combine nanoparticles with immunotherapy. The complexity in the multiple and possibly contradictory effects on immune cells, especially when using encapsulated cytotoxics, require finely tuned protocols in terms of dosing, sequencing and treatment duration. With respect to the number of treatment modalities and scheduling, extensive mathematical resources are required to help determining *in silico* the strategy that is the most efficient to yield synergistic effect between nanoparticles, immune check-point inhibitors, and possibly other drugs likely to boost efficacy such as anti-angiogenics. Should the optimal conditions be met, because nanoparticles are more protective towards hematopoietic progenitors and therefore less likely to trigger lymphopenia, not to mention stroma-

targeting properties and boosting tumor immunogenicity, they could be of great value for awakening tumor immunity and help once resistant solid tumors to respond to immune check-point inhibitors?

Article highlights

- Immunotherapy is currently limited by cold tumors displaying unfavorable immunogenic profile because of low mutational burden, harsh tumor micro-environment, plus possible deleterious impact of prior chemotherapy on patient's innate immunity.
- Combinatorial therapy is seen as the future of immunotherapy, and associated treatments are all expected to turn cold tumors into hot tumors, so as to boost the efficacy of immune check-point inhibitors.
- Nanoparticles present promising features, making them particularly suitable candidates to be associated with immune check-point inhibitors.
- Improved pharmacokinetics, immunogenic properties and ability to cargo a wide variety of anticancer drugs or immunomodulating agents provide nanoparticles with unique properties.
- Determining the optimal modality of such combination is challenging because of the multiple and complex interplays between nanoparticles, patient's immunity, tumor micro-environment and tumor cells.

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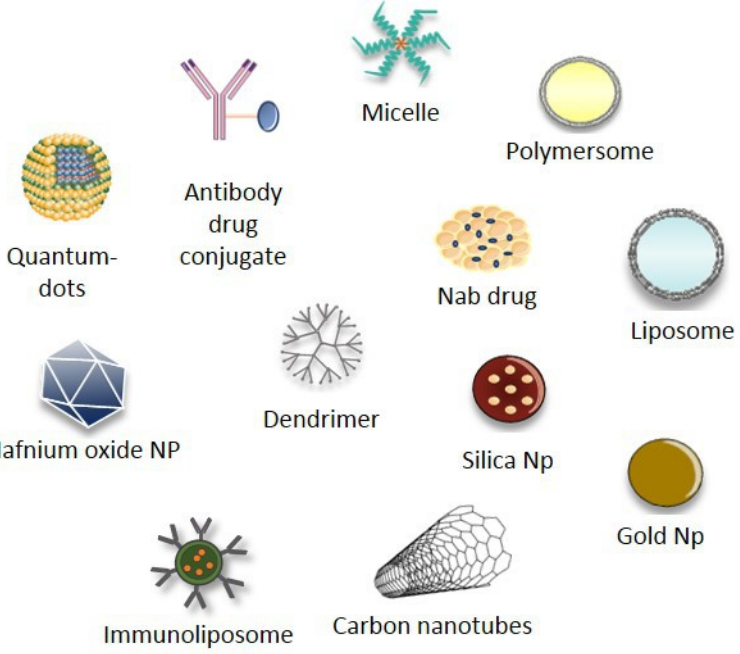
Figures Legends

Figure 1: Different scaffolds and nanoparticles available to cargo payloads to the tumor site. NP: nanoparticle.

Figure 2: Schematic comparison of the biodistribution profiles of xenobiotics administered as free drugs (A) or as nanoparticles (B).

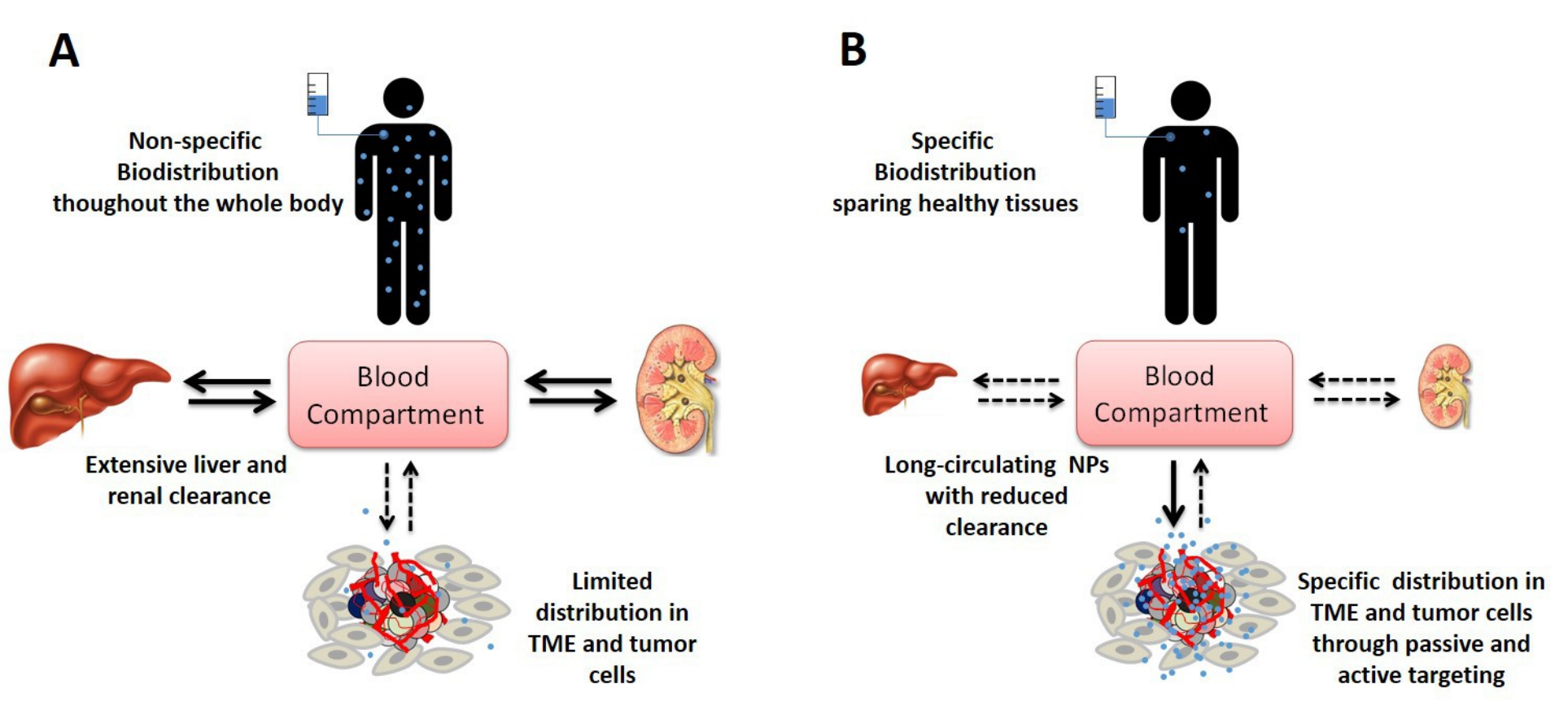
Figure 3: Representation of the possible multiple interplays between nanoparticles, cancer cells, and immune cells in the TME. TAM: Tumor-Associated Macrophages. MDSC: Myeloid Derived Suppressive Cells, APC: Antigen-Presenting Cells.

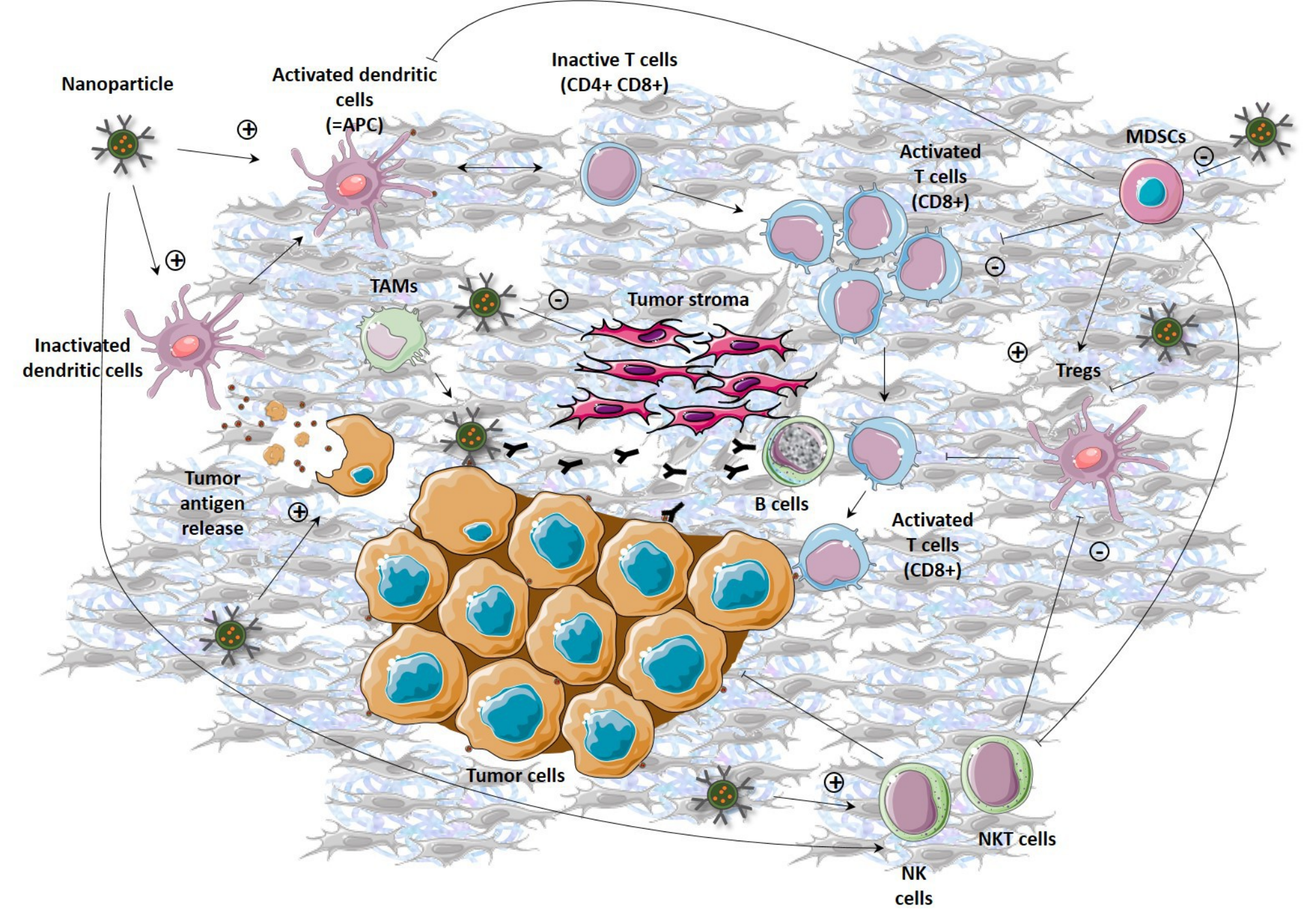


CARRIERS

CARRIED PAYLOAD

- Antibodies
- Cytotoxics
- Cytokines
- Imaging agents
- RNA
- Cancer vaccine


A
Non-specific
Biodistribution
thoughout the whole body
Blood
Compartment
Extensive liver and
renal clearance
Limited
distribution in
TME and tumor
cells
B
Specific
Biodistribution
sparing healthy tissues
Blood
Compartment
Long-circulating NPs
with reduced
clearance
Specific distribution in
TME and tumor cells
through passive and
active targeting


Nanoparticle
Activated dendritic cells (=APC)
Inactive T cells (CD4+ CD8+)
MDSCs
Activated T cells (CD8+)
TAMs
Tumor stroma
Inactivated dendritic cells
Tregs
Tumor antigen release
B cells
Activated T cells (CD8+)
Tumor cells
NKT cells
NK cells